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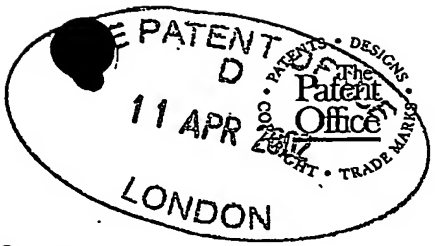

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2. Patent application number *(The Patent Office will fill in this part)* — 0208390.5

3. Full name, address and postcode of the or of each applicant *(underline all surnames)* — UNIVERSITY COLLEGE LONDON
GOWER STREET
LONDON WC1E 9BT

Patents ADP number *(if you know it)* — 40968970001

If the applicant is a corporate body, give the country/state of its incorporation — UNITED KINGDOM

4. Title of the invention — ADENO-ASSOCIATED VIRUS PRODUCER SYSTEM

5. Name of your agent *(if you have one)* — J.A. KEMP & CO.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* — 14 South Square
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London
WC1R 5JJ

Patents ADP number *(if you know it)* — 26001

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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WOODS, Geoffrey Corlett
020 7405 3292

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ADENO-ASSOCIATED VIRUS PRODUCER SYSTEM

Field of the Invention

The present invention relates to the use of a herpes helper virus in the production of adeno-associated virus (AAV) vectors, to a novel herpes helper virus and to an improved method of producing AAV vectors using a herpes helper virus.

Background to the Invention

Adeno-associated virus (AAV) vectors are promising for gene delivery and gene therapy in a number of target tissues including muscle, liver and brain. AAV is a naturally defective virus which requires a helper adenovirus or herpes virus for growth. AAV vectors are versions of AAV in which the genes encoding the necessary replication (rep) and structural (cap) proteins have been deleted to allow insertion of the sequences to be delivered between the remaining terminal repeat sequences. For growth of vectors therefore, not only is a helper virus required, but the genes encoding the rep and cap proteins must be delivered to or be present in the cells used for production to provide the rep and cap functions *in trans*.

Such production systems have provided a problem in the field because stable cell lines expressing appropriate levels of rep and cap genes have been difficult to produce. Versions of helper adenovirus containing these genes have also not solved the problem. Thus in general for the production of AAV vectors, a plasmid encoding the rep and cap genes and a plasmid containing the DNA sequence to be packaged flanked by AAV terminal repeats are transfected into cells and then the cells infected with a helper adenovirus. This process for the production of AAV vectors is relatively laborious and inefficient and is hard to scale up.

AAV production systems using herpes simplex virus (HSV) as a helper virus are also known. An amplicon system has been described but the use of HSV as a helper virus for AAV growth has to date been best accomplished using non-replicating mutants of HSV deleted for ICP27 (Conway *et al* 1999).

Summary of the Invention

The present inventors have devised a novel AAV production system which uses as a helper virus a herpes virus, preferably a herpes simplex virus (HSV), having improved properties compared to previous herpes helper viruses. When HSV is used, the novel AAV production system of the present invention uses a HSV helper virus containing an ICP27 protein which allows HSV replication to occur but which shows reduced inhibition of mRNA splicing compared to wild-type HSV-1 ICP27. The ICP27 protein may be a mutant protein which is mutated so that the usual inhibition of mRNA splicing associated with expression of ICP27 is reduced or prevented, but so that virus replication is not prevented. Alternatively, a non-HSV homologue of ICP27 with these properties may be used in the producer system of the invention.

HSV genes are in the main non-spliced while the majority of mammalian cellular genes are spliced. HSV has evolved a mechanism of enhancing the expression of HSV genes as compared to host genes by inhibiting mRNA splicing. This effect is largely mediated through the HSV ICP27 protein, a multi-functional essential gene required for virus growth and replication. The AAV rep and cap genes are multiply spliced and this splicing will therefore be inhibited in the presence of wild type HSV.

The use of a HSV helper virus capable of replicating and having a reduced property of inhibiting the splicing of mRNA in infected cells can enable an increased efficiency of production of AAV vectors to be achieved compared to the use of non-replicative HSV helper viruses which have been found to be most effective previously.

Accordingly, the present invention provides:

the use of a replication competent herpes virus which

(a) lacks a functional wild-type HSV ICP27 gene; and

(b) comprises an ICP27 gene encoding an ICP27 protein which allows replication of said herpes virus to occur and which has a reduced ability to inhibit RNA splicing compared to wild-type HSV ICP27

in the production of an adeno-associated virus (AAV) vector;

- a replication competent herpes virus which
  (a) lacks a functional wild-type HSV ICP27 gene;
  (b) comprises an ICP27 gene encoding an ICP27 protein which allows replication of said herpes virus to occur and which has a reduced ability to inhibit RNA splicing compared to wild-type HSV ICP27; and
  (c) comprises AAV rep and/or cap genes and/or an AAV vector sequence;
- a method of producing an AAV vector comprising:
  (i) introducing into producer cells:
    (a) a herpes virus which lacks a functional wild-type HSV ICP27 gene;
    (b) an ICP27 gene encoding an ICP27 protein which allows replication of said herpes virus to occur and which has a reduced ability to inhibit RNA splicing compared to wild-type HSV ICP27;
    (c) AAV rep and cap genes; and
    (d) an AAV vector sequence; and
  (ii) isolating the AAV vector particles produced;
- an AAV vector produced by a method of the invention;
- a pharmaceutical composition comprising an AAV vector according to the invention and a pharmaceutically acceptable carrier or diluent;
- a method of producing a pharmaceutical composition comprising formulating an AAV vector according to the invention with a pharmaceutically acceptable carrier or diluent;
- a method of gene therapy comprising administering a therapeutically effective amount of an AAV vector according to the invention to a patient in need thereof; and

a kit for producing an AAV vector comprising:

(a) a replication competent herpes virus which lacks a functional wild-type HSV ICP27 gene;

(b) an ICP27 gene encoding an ICP27 protein which allows replication of said herpes virus to occur and which has a reduced ability to inhibit RNA splicing compared to wild-type HSV ICP27;

(c) AAV rep and cap genes;

(d) an AAV vector sequence; and optionally

(e) producer cells

wherein (b), (c) and/or (d) are incorporated into said herpes virus (a), are present on separate plasmids or are stably integrated into said producer cells (e).

Detailed Description of the Invention

The invention provides the use of a replication competent herpes virus which

(a) lacks a functional wild-type HSV ICP27 gene; and

(b) comprises an ICP27 gene encoding an ICP27 protein which allows replication of said herpes virus to occur and which has a reduced ability to inhibit RNA splicing compared to wild-type HSV ICP27

in the production of an adeno-associated virus (AAV) vector.

A herpes virus which lacks a functional wild-type HSV ICP27 may be an HSV, preferably HSV-1, HSV-2 or an intertypic recombinant HSV, in which the wild-type HSV ICP27 gene has been deleted or in which the HSV ICP27 gene has been mutated such that one or more of the normal functions of the ICP27 protein is inhibited or prevented. Alternatively the herpes virus may be a non-HSV herpes virus which naturally does not contain a wild-type HSV ICP27 gene or a non-HSV herpes virus which lacks a functional wild-type HSV ICP27 homologue.

By a "homologue" it is meant a herpes virus gene that performs the same or a similar function as ICP27 in HSV and that exhibits sequence homology, at the amino acid level, to the HSV ICP27 protein. Typically, a homologue of, the HSV ICP27 gene will show at least 15%, preferably at least 20%, sequence identity at the amino acid level to the HSV ICP27 gene over a region of at least 20, preferably at least 30, for instance at least 40, 60 or 100 or more contiguous amino acids.

Methods of measuring protein and nucleotide homology are well known in the art and it will be understood by those of skill in the art that in the present context, protein homology is calculated on the basis of amino acid identity (sometimes referred to as "hard homology").

Methods of measuring nucleic acid and protein homology are well known in the art. For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings) (Devereux *et al.* (1984) *Nucleic Acids Research* **12**, p387-395). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (typically on their default settings), for example as described in Altschul (1993) J. Mol. Evol. 36:290-300; Altschul *et al.* (1990) J. Mol. Biol. 215:403-10.

Software for performing BLAST analyses is publicly available through the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al.*, 1990). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of

the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci.* USA 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) *Proc. Natl. Acad. Sci.* USA 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

Homologues of the HSV ICP27 gene from other herpes viruses can be identified in a number of ways, for example by probing genomic or cDNA libraries made from the herpes virus with probes comprising all or part of the HSV ICP27 gene under conditions of medium to high stringency (0.2X SSC/0.1% SDS at from about 40°C to about 55°C). Alternatively, species homologues may also be obtained using degenerate PCR which will use primers designed to target sequences within the homologues encoding conserved amino acid sequences. The primers will contain one or more degenerate positions and will be used at stringency conditions lower than those used for cloning sequences with single sequence primers against known sequences (for example, 2 x SSC at 60°C).

The wild-type HSV ICP27 gene or wild-type ICP27 homologue may be rendered functionally inactive by several techniques well known in the art. For example, they may be rendered functionally inactive by deletion(s), substitution(s) or insertion(s), preferably by deletion. Deletions may remove portions of the genes or the entire gene. For example, deletion of only one nucleotide may be made, resulting in a frame shift. However, preferably larger deletions are made, for example at least 25%, more preferably at least 50% of the total coding and non-coding sequence (or alternatively, in absolute terms, at least 10 nucleotides, more preferably at least 100

nucleotides, most preferably, at least 1000 nucleotides). It is particularly preferred to remove the entire gene and some of the flanking sequences. Inserted sequences may include the mutated ICP27 gene or homologous ICP27 gene described below.

Mutations may be made in the herpes viruses by homologous recombination methods well known to those skilled in the art. For example, HSV genomic DNA is transfected together with a vector, preferably a plasmid vector, comprising the mutated sequence flanked by homologous HSV sequences. The mutated sequence may comprise deletion(s), insertion(s) or substitution(s), all of which may be constructed by routine techniques. Insertions may include selectable marker genes, for example lacZ, for screening recombinant viruses by, for example, β-galactosidase activity.

An ICP27 gene encoding an ICP27 protein which protein allows herpes virus replication to occur and which has a reduced ability to inhibit RNA splicing compared to wild-type HSV ICP27 is as defined as a nucleotide sequence comprising a promoter sequence operably linked to a sequence encoding an ICP27 protein with the required properties. Any suitable promoter sequence may be used, for example an immediate early gene promoter. Preferably the promoter sequence is an ICP27 promoter, such as the HSV-1 or HSV-2 ICP27 promoter. More preferably, the promoter is the endogenous ICP27 promoter in HSV or the endogenous promoter of an ICP27 homologue in a non-HSV vector.

The ICP27 protein in the herpes virus may be an HSV ICP27 protein which contains a mutation that prevents the ICP27 protein from inhibiting RNA splicing or which reduces the level of inhibition of RNA splicing compared to wild-type HSV ICP27 protein. The mutation of the ICP27 protein does not prevent replication of the herpes virus.

A herpes helper virus of the invention has a reduced property of inhibiting the splicing of mRNA in infected cells. Inhibition of RNA splicing by the ICP27 protein may be reduced by 10, 20, 30 or 40%, preferably by 50, 60, 70, 80 or 90% compared to wild-type HSV ICP27 protein, for example to wild-type HSV-1 or HSV-2 ICP27 protein. Any suitable assay for monitoring RNA splicing may be used to determine whether an ICP27 protein has a reduced ability to inhibit RNA splicing.

A herpes helper virus of the invention thus allows more mRNA splicing to occur in infected cells than can occur in cells infected with a herpes virus comprising a wild-type HSV ICP27 gene. Preferably the herpes virus of the invention allows a significant amount of mRNA splicing to occur in infected cells, i.e. the inhibition of gene splicing the ICP27 protein encoded by the herpes helper virus is negligible. RNA splicing in cells infected with a virus of the invention may be uninhibited or be inhibited by 1, 5, 10, 20, 30, 40, 50, 60, 70 or 80% compared to RNA splicing in cells not infected with a herpes virus.

The ICP27 gene may be mutated so that its ability to inhibit gene splicing is impaired by any technique well known in the art, for example, by deletion(s), substitution(s) or insertion(s) into the portion(s) of the gene important for the inhibition of gene splicing. It is not desirable to delete large portions of the ICP27 gene because it is necessary that the gene retains its ability to enable herpes virus replication to occur. However, small deletions, insertions and/or substitutions may be made as appropriate to diminish the gene splicing-inhibitory activity. Examples of mutant ICP27 proteins are described in Soliman *et al.* 1997.

Alternatively, the ICP27 protein may be an ICP27 homologue from a non-HSV herpes virus, which homologue does not inhibit RNA splicing, or shows reduced inhibition of RNA splicing compared to wild-type HSV ICP27 protein, and which does not prevent the herpes virus from replicating.

The herpes virus may additionally comprise AAV rep and/or cap genes. The rep and/or cap genes are preferably inserted into a site or sites of the herpes virus genome such that replication of the herpes virus is not prevented. Suitable sites of insertion include the UL43, US5 and LAT loci but other insertion sites may also be used. The rep and cap genes comprise the coding sequences of the rep and cap genes from an AAV operably linked to control sequences. Any suitable control sequences may be used. For example, the rep and cap genes may be under the control of herpes virus promoters. Preferably, the control sequences are the promoters which usually control rep and cap expression in AAV.

The herpes virus may comprise an AAV vector sequence. The AAV vector sequence is preferably inserted into a site or sites of the herpes virus genome such

that replication of the herpes virus is not prevented. Suitable sites of insertion include the UL43, US5 and LAT loci but many other insertion sites may also be used.

The AAV vector sequence is typically a DNA sequence to be packaged flanked by AAV terminal repeats. The DNA sequence to be packaged is generally an expression cassette comprising a sequence encoding a heterologous protein operably linked to a control sequence. It is preferred that the heterologous protein is a therapeutic protein.

The invention also provides a herpes virus which

(a) lacks a functional wild-type HSV ICP27 gene;

(b) comprises an ICP27 gene encoding an ICP27 protein which permits replication of said herpes virus and which has a reduced ability to inhibit RNA splicing compared to wild-type HSV ICP27;

(c) an AAV cap gene and/or an AAV rep gene; and/or

(d) an AAV vector sequence.

A herpes virus of the invention is preferably HSV-1 or HSV-2 or an intertypic recombinant virus containing DNA from HSV-1 and HSV-2 strains. A herpes virus of the invention may be a non-HSV virus which further lacks a functional wild-type HSV ICP27 homologue. The ICP27 gene (b) and the AAV sequences (c) and (d) are as defined above.

A further aspect provided by he present invention is a method of producing an AAV vector comprising:

(i) introducing into producer cells:

(a) a herpes virus which lacks a functional wild-type HSV ICP27 gene;

(b) an ICP27 gene encoding an ICP27 protein which allows herpes virus replication to occur and which has a reduced ability to inhibit RNA splicing compared to wild-type HSV ICP27;

(c) AAV rep and cap genes; and

(d) an AAV vector sequence; and

(ii) isolating the AAV vector particles produced.

The ICP27 gene encoding an ICP27 protein (b) is as defined above. The ICP27 gene may be incorporated into the herpes virus (a). Alternatively, the ICP27 gene is not incorporated into the herpes virus but is present in the producer cells such that it can complement the lack of HSV ICP27 gene in the herpes virus *in trans*. The ICP27 gene may be introduced into the producer cells by transient transfection of an plasmid into the producer cells either before or after the cells are infected with HSV. In a preferred embodiment of the invention the ICP27 gene is stably integrated into the genome of the producer cells. Suitable methods of transient and stable transfection are well known in the art.

Where the ICP27 gene is introduced into the producer cells by transient or stable transfection, there is preferably no overlap between the nucleotide sequences in the plasmid or cell line and the nucleotide sequences of the herpes virus, thus preventing homologous recombination. Prevention of homologous recombination is important in order to prevent the generation of a herpes virus which can replicate in the absence of the producer cells. The AAV particles produced by such a method will therefore be free from any contaminating replication competent herpes virus.

The AAV rep and/or cap genes (c) and/or the AAV vector sequence (d) may be incorporated into the herpes virus (a). The rep and/or cap genes and/or AAV vector sequences are preferably inserted into a site or sites of the herpes virus genome such that replication of the herpes virus is not prevented. Suitable sites of insertion include the UL43, US5 and LAT loci but many other insertion sites may also be used.

Alternatively the AAV rep and/or cap genes (c) and/or the AAV vector sequence (d) may each be introduced into the cells by transient or stable transfection of suitable plasmids. Suitable methods of transfection are well known in the art. When transient transfection is used to introduce the AAV rep and/or cap genes and/or the AAV vector sequence into the producer cells, the transfection may be carried out before or after infection of the cells with the herpes virus. If stable transfection is used, it is preferable that the AAV rep and/or cap genes and/or AAV

vector sequences are integrated into the producer cell genome prior to infection of the producer cells with the herpes virus.

Producer cells used in the invention include any cell line that supports the growth of the herpes virus. A suitable cell line is a cell line which hosts herpes viruses and forms colonies. Typically the cell line is a mammalian cell line such as a rodent or human cell line. A particularly preferred cell line is based on BHK or Vero cells.

In a further aspect, the present invention provides a kit for producing an AAV vector comprising:

(a) a replication competent herpes virus which lacks a functional wild-type HSV ICP27 gene;

(b) an ICP27 gene encoding an ICP27 protein which allows herpes virus replication to occur and which has a reduced ability to inhibit RNA splicing compared to wild-type HSV ICP27;

(c) AAV rep and cap genes;

(d) an AAV vector sequence; and optionally

(e) producer cells

wherein (b), (c) and/or (d) are incorporated into said herpes virus (a), are present on separate plasmids or are stably integrated into said producer cells (e).

In a further aspect of the invention is provided an AAV vector produced by a method of the invention, produced using a herpes virus of the invention or using a kit of the invention. The AAV vector is typically lacking rep and cap genes and comprises a heterologous gene, i.e. a sequence encoding a heterologous protein, operably linked to control sequences. The heterologous protein is preferably a therapeutic protein. A therapeutic protein is defined herein as being a protein which it is desirable to introduce into a cell or cells of a patient in order to treat a disease or disorder.

An AAV vector of the invention may be formulated with a pharmaceutically acceptable carrier or diluent and/or may be administered to a patient in a method of treatment of a disease or disorder, for example by gene therapy.

Accordingly, the present invention provides a pharmaceutical composition for use in a method of treatment such as gene therapy comprising an AAV vector according to the invention and a pharmaceutically acceptable carrier or diluent. A method of producing a pharmaceutical composition by admixing an AAV vector of the invention with a pharmaceutically acceptable carrier or diluent is also provided.

A method of treatment of the human or animal body, for example a method of gene therapy, comprising administering a therapeutically effective amount of an AAV vector of the invention to a patient in need thereof. A therapeutically effective amount of an AAV vector is an amount which enables a therapeutic gene in the vector to be expressed in cells of a patient at a level that alleviates the symptoms of the disease or disorder to be treated or that improves the condition of the patient. A patient in need of treatment is a patient suffering from or predisposed to a disease or disorder that can be treated by administration of a therapeutic gene incorporated into an AAV vector.

The invention is illustrated by the following preferred embodiments:

Embodiment 1

(i) The HSV helper virus has the wild type HSV ICP27 gene replaced with a mutant version of ICP27 with which the inhibition of splicing is reduced but which can still support the replication of HSV. Examples of such mutations have been reported previously (Soliman *et al.* 1997). Alternatively the wild type ICP27 gene is replaced with a non-HSV homologue of ICP27 which supports virus growth but which displays a reduced inhibition of splicing as compared to HSV ICP27;

(ii) Plasmids encoding the AAV vector sequences and the AAV rep and cap genes are stably or transiently transfected into cells which can support the growth of HSV. In the case of stable transfection, these cells are then infected with helper viruses as described in (i) above. In the case of transient transfection, cells are infected with the helper virus before, at the same time as, or after transfection. Experiment will determine the optimal timing for this;

(iii) AAV vector particles are produced.

Embodiment 2

(i) The HSV helper virus has the wild type HSV ICP27 gene replaced with a mutant version of ICP27 with which the inhibition of splicing is reduced but which can still support the growth of HSV. Examples of such mutations have been reported previously (Soliman *et al.* 1997). Alternatively the wild type ICP27 gene is replaced with a non-HSV homologue of ICP27 which supports virus growth but which displays a reduced inhibition of splicing as compared to HSV ICP27;

(ii) Any of the AAV rep, cap or vector sequences to be packaged are incorporated into the helper virus described above, reducing the number of plasmids required to be stably or transiently transfected into the producer cells, prior to use for AAV vector production; and

(iii) AAV vector particles are produced

In a particularly preferred version of embodiment 2, the helper virus is an HSV in which the wild-type HSV ICP27 gene has been replaced with a mutant HSV-1 ICP27 gene. The mutant HSV-1 ICP27 gene is capable of supporting virus growth but shows a reduced inhibition of splicing compared to wild type HSV-1 ICP27 is constructed. The mutant HSV-1 ICP27 gene may be a mutant as described in Soliman *et al.* 1997. The rep and cap encoding sequences from AAV are inserted into the HSV helper virus. The site or sites of insertion of the rep and cap genes is chosen so that HSV replication is not prevented. Suitable sites include the UL43, US5 or LAT loci, but many others are possible. The HSV helper virus is used to infect cells, such as BHK or Vero cells, which support the growth of HSV in which the AAV vector sequence is stably integrated into the cellular genomic DNA. AAV vector particles are then produced.

In a second preferred version of embodiment 2, the HSV helper virus is identical to that used in the first preferred version except that in addition to the rep and cap encoding sequences from AAV, the AAV vector sequence is inserted into the HSV helper virus. The site of insertion of the AAV vector sequence is chosen so

that HSV replication is not prevented. Suitable sites include the UL43, US5 or LAT loci, but many others are possible. The HSV helper virus is used to infect cells, such as BHK or Vero cells, which support the growth of HSV. AAV vector particles are then produced.

Embodiment 3

(i) The HSV helper virus has the wild type HSV ICP27 gene mutated such that no replication is possible in cells which do not complement the deficiency.

(ii) The cells used for production contain a version of ICP27 with which the inhibition of splicing is reduced but which can still support the growth of HSV introduced by stable or transient transfection. Alternatively a non-HSV homologue of ICP27 which supports virus growth but which displays a reduced inhibition of splicing as compared to HSV ICP27 is used;

(iii) AAV rep and cap and vector sequences are introduced and helper virus provided as in Embodiments 1 or 2 above; and

(iv) AAV vector particles are produced.

References

Conway JE *et al.*, 1999, Gene Therapy 6, 986-993

Soliman TM *et al.*, 2000, J. Virology 71, 9188-9197

CLAIMS

1. Use of a replication competent herpes virus which

(a) lacks a functional wild-type HSV ICP27 gene; and

(b) comprises an ICP27 gene encoding an ICP27 protein which allows replication of said herpes virus to occur and which has a reduced ability to inhibit RNA splicing compared to wild-type HSV ICP27

in the production of an adeno-associated virus (AAV) vector.

2. Use according to claim 1 wherein said herpes virus is HSV-1 or HSV-2.

3. Use according to claim 1 or 2 wherein said ICP27 protein is a non-HSV homologue of ICP27.

4. Use according to any one of the preceding claims wherein said ICP27 protein is a mutant protein.

5. Use according to claim 4 wherein the herpes virus is not an HSV and wherein the herpes virus lacks a functional wild-type HSV ICP27 homologue.

6. Use according to any one of the preceding claims wherein the herpes virus further comprises AAV rep and cap genes.

7. Use according to any one of the preceding claims wherein the herpes virus further comprises an AAV vector sequence.

8. Use according to claim 6 or 7 wherein said AAV rep and cap genes and/or said AAV vector sequence are inserted into the UL43 locus, US5 locus or LAT locus of said herpes virus.

9. A replication competent herpes virus as defined in any one of claims 6 to 8.

10. A method of producing an AAV vector comprising:

(i) introducing into producer cells:

(a) a herpes virus which lacks a functional wild-type HSV ICP27 gene;

ABSTRACT

ADENO-ASSOCIATED VIRUS PRODUCER SYSTEM

The present invention provides the use of a replication competent herpes virus which

(a) lacks a functional wild-type HSV ICP27 gene; and

(b) comprises an ICP27 gene encoding an ICP27 protein which allows replication of said herpes virus to occur and which has a reduced ability to inhibit RNA splicing compared to wild-type HSV ICP27

in the production of an adeno-associated virus (AAV) vector.

(b) an ICP27 gene encoding an ICP27 protein which allows replication of said herpes virus to occur and which has a reduced ability to inhibit RNA splicing compared to wild-type HSV ICP27;

(c) AAV rep and cap genes; and

(d) an AAV vector sequence; and

(ii) isolating the AAV vector particles produced.

11. A method according to claim 10 wherein said herpes virus (a) comprises said ICP27 gene (b).

12. A method according to claim 10 wherein said ICP27 gene (b) is stably or transiently infected into said producer cells.

13. A method according to any one of claims 10 to 12 wherein said AAV rep and cap genes (c) and/or said AAV vector sequence (d) are inserted into said herpes virus (a).

14. A method according to claim 13 wherein said AAV rep and cap genes (c) and/or said AAV vector sequence (d) are inserted into the UL43 locus, US5 locus or LAT locus of said herpes virus.

15. A method according to any one of claims 10 to 12 wherein said AAV rep and cap genes and/or said AAV vector sequence (d) are stably or transiently transfected into said producer cells.

16. A method according to claim 12 or 15 wherein said producer cells are stably transfected prior to infection with said herpes virus (a).

17. A method according to claim 12 or 15 wherein said producer cells are transiently transfected before infection with said herpes virus (a).

18. A method according to claim 12 or 15 wherein said producer cells are transiently transfected after infection with said herpes virus (a).

19. A method according to any one of claims 10 to 15 wherein the producer cells are BHK or Vero cells.

20. An AAV vector produced by a method of any one of claims 10 to 19.

21. A pharmaceutical composition comprising an AAV vector according to claim 20 and a pharmaceutically acceptable carrier or diluent

22. A method of producing a pharmaceutical composition comprising mixing an AAV vector according to claim 20 with a pharmaceutically acceptable carrier or diluent.

A method of producing a pharmaceutical composition comprising carrying out the method of any one of claims 10 to 19 and formulating said isolated AAV vector particles with a pharmaceutically acceptable carrier or diluent.

23. A method of gene therapy comprising administering a therapeutically effective amount of an AAV vector according to claim 20 to a patient in need thereof.

24. A kit for producing an AAV vector comprising:

(a) a replication competent herpes virus which lacks a functional wild-type HSV ICP27 gene;

(b) an ICP27 gene encoding an ICP27 protein which allows replication of said herpes virus to occur and which has a reduced ability to inhibit RNA splicing compared to wild-type HSV ICP27;

(c) AAV rep and cap genes;

(d) an AAV vector sequence; and optionally

(e) producer cells

wherein (b), (c) and/or (d) are incorporated into said herpes virus (a), are present on separate plasmids or are stably integrated into said producer cells (e).

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